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Revised Test Plan for
C.I. Acid Yellow 23
CAS No. 1934-21-0

Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:
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Colorcon

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Sensient Colors, Inc.

.

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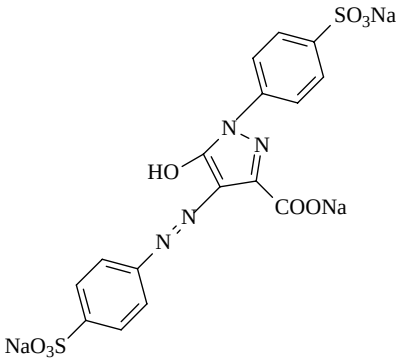
Test Plan for C.I. Acid Yellow 23

1 IDENTITY OF SUBSTANCES



C.I. Acid Yellow 23

CAS No. 1934-21-0

Synonyms:
FD&C Yellow No. 5
Tartrazine

2 CATEGORY ANALYSIS

2.1 INTRODUCTION

The International Association of Color Manufacturers (IACM) has volunteered to participate in the EPA's Chemical "Right-to-Know" Program. IACM is committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing on the chemicals used by the color industry in order to assure their human and environmental safety. The category analysis, test plan, and robust summaries presented represent the first phase of IACM's commitment to the Chemical "Right-to-Know" Program.

2.2 BACKGROUND INFORMATION

This category analysis and test plan provides data for FD&C Yellow No. 5. FD&C Yellow No. 5 is used as a food, drug, and cosmetic colorant. It is used to color candies and confections, bakery goods, cakes, cookies, ice cream, sherbets, cereals, soft drinks, sausage casings, jams and jellies, gelatin and pudding powders, beverage powders, maraschino cherries, prepared meats, canned and frozen vegetables, animal feeds, aqueous drug solutions, tablets, capsules, toothpastes, hair-waving fluids, bath salts, hair rinses, and printing inks for use in and on foods, drugs, and cosmetics and on food, drug, and cosmetic packaging materials.

FD&C Yellow No. 5 is an azo dye. Azo compounds are formed from arenediazonium ions reacting with highly reactive aromatic compounds, in what is called a diazo coupling reaction. Azo compounds are generally deeply colored because the azo linkage brings the two aromatic rings into conjugation [Solomon, 1996]. In addition to possessing extended conjugation, many azo dyes are also ring substituted with sulfonic acid substituents, which significantly increase polarity and water solubility and decrease absorption *in vivo*.

2.3 REGULATORY STATUS

FD&C Yellow No. 5 is a certified color additive approved in the United States to color food, drugs and cosmetics. Certified color additives are synthetic organic compounds that must meet high purity specifications established by the Food and Drug Administration (FDA) (see Table 1 below). Each batch of manufactured certified color in the United States is tested by the FDA for compliance with these specifications [Frick and Meggos, 1988]. Certified color additives are among the most thoroughly studied of all food ingredients because of the rigorous testing for human health endpoints required by the 1960 Color Additive Amendments to the FD&C Act [Hallagan, 1991]. There are currently only seven certified color additives approved for food, drug and cosmetic use in the United States.

Table 1. US FDA Specifications

FD&C Yellow No. 5 shall conform to the following specifications and shall be free from impurities other than those named to the extent that such other impurities may be avoided by good manufacturing practice (21 CFR 74.705):

• Sum of volatile matter at 135° C (275°F) and chlorides and sulfates (calculated as sodium salts), not more than 13 percent.

• Water-insoluble matter, not more than 0.2 percent.

• 4,4'-[4,5-Dihydro-5-oxo-4-[(sulfophenyl)hydrazono]-1H-pyrazol-1,3-diyl bis[benzenesulfonic acid], trisodium salt, not more than 1 percent.

• 4[(4',5-Disulfo[1,1'-biphenyl]-2-yl)hydrazono]]-4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazole-3carboxylic acid, tetrasodium salt, not more than 1 percent.

• Ethyl or methyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl) hydrazono] 1H-pyrazole-3-carboxylate, disodium salt, not more than 1 percent.

• Sum of 4,5-dihydro-5-oxo-1-phenyl-4-[(4-sulfophenyl)azo]-1H-pyrazole -3- carboxylic acid, disodium salt, and 4,5-dihydro-5-oxo-4-(phenylazo)-1-(4-sulfophenyl)-1H-pyrazole- 3- carboxylic acid, disodium salt, not more than 0.5 percent.

• 4-Aminobenzenesulfonic acid, sodium salt, not more than 0.2 percent.

• 4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazole-3-carboxylic acid, disodium salt, not more than 0.2 percent.
• Ethyl or methyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazole -3-carboxylate, sodium salt, not more than 0.1 percent.

• 4,4'-(1-Triazene-1,3-diyl)bis[benzenesulfonic acid], disodium salt, not more than 0.05 percent.
- 4-Aminoazobenzene, not more than 75 parts per billion.
- 4-Aminobiphenyl, not more than 5 parts per billion.
- Aniline, not more than 100 parts per billion.
- Azobenzene, not more than 40 parts per billion.
- Benzidine, not more than 1 part per billion.
- 1,3-Diphenyltriazene, not more than 40 parts per billion.
- Lead (as Pb), not more than 10 parts per million.
- Arsenic (as As), not more than 3 parts per million.
- Mercury (as Hg), not more than 1 part per million.
- Total color, not less than 87 percent.

FD&C Yellow No. 5 was first listed for food use in the United States in 1916. In 1994, 799,531.4 kg of FD&C Yellow No. 5 dye and 441,000.9 kg of FD&C Yellow No. 5 lake were certified for use in the United States.

The World Health Organization/Food and Agriculture Organization Joint Expert Committee for the Evaluation of Food Additives (WHO/FAO JECFA) has also evaluated the safety of FD&C Yellow No. 5 used as a coloring agent in food. An average daily intake (ADI) of 0-7.5 mg/kg bw per day was assigned by JECFA in 1964 based on the extensive human toxicological information available that indicated FD&C Yellow No. 5 did not possess carcinogenic potential (see Table 2 below).

Table 2. Regulatory Approvals/Consumption Limits[1]	
USA	GMP (21 CFR 74.705)
EEC	GMP (EC Journal No. L237/13; 1994)
JECFA	ADI of 0-7.5 mg/kg (8th report, 1964)

Based on the long history of use of FD&C Yellow No. 5 in food, the many hazard assessments performed by the United States FDA and WHO/FAO JECFA, and the current regulatory status of FD&C Yellow No. 5, there is no compelling evidence that this substance should be further tested for human health endpoints in the EPA Chemical "Right to Know" Program.

2.4 STRUCTURAL CLASSIFICATION

FD&C Yellow No. 5 is principally the trisodium salt of 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[4-sulfophenyl-azo]-1H-pyrazole-3-carboxylic acid (USFDA-21 CFR 74.705).

2.5 INDUSTRIAL PRODUCTION

In order to manufacture FD&C Yellow No. 5, 4-amino-benzenesulfonic acid is diazotized using hydrochloric acid and sodium nitrite. The diazo compound is then coupled with 4,5-dihydro-5-oxo-1-(4sulfophenyl)-1H-pyrazole-3-carboxylic acid or with the methyl ester, the ethyl ester, or a salt of this carboxylic acid. The resulting dye is purified and isolated as the sodium salt.

[1] IACM, 2003

2.6 PHARMACOKINETICS AND METABOLISM

FD&C Yellow No. 5 undergoes bacterial azo reduction in the gastrointestinal tract of rats, rabbits, and humans [Allan & Roxon, 1974; Chung *et al*., 1978; Dubin & Wright, 1975; Roxon *et al*., 1967a; Roxon *et al*., 1967b; and Watabe *et al*., 1980]. Following reductive cleavage of the azo linkage by intestinal bacteria, sulfanilic acid and aminopyrazolone are produced. The pyrazolone fragment is further degraded by intestinal bacteria to yield a second molecule of sulfanilic acid. In rats, relatively small amounts of these metabolites are excreted in the urine with the majority being detected in the feces [Honohan *et al*., 1977].

Groups of Sprague-Dawley female rats were given single oral doses of aqueous solutions (1%) containing 2 to 25 mg of ^{14}C-tartrazine labeled in the 1-p-sulphophenyl ring. Urine and feces were collected at 24-hour intervals. Bile was collected from bile duct cannulated animals and blood was collected regularly from the orbital sinus. After 72 hours, animals were sacrificed an tissues from the liver, spleen, kidneys, stomach, small intestine, caecum, large intestine, and peri-uterine fat sample were subjected for radioassay. Total 72-hour urinary excretion of tartrazine was only 4.0%. Biliary excretion was less than 0.1% while there was only trace amounts of radioactivity in internal organs after 72 hours. In terms of metabolites, 21% of the total radioactivity was detected in the urine as sulfanilic acid. Twenty-four hours after dosing, approximately equal amounts of urine radioactivity (43-44%) was accounted for by sulfanilic acid and aminopyrazolone. The urinary radioactivity corresponded to 20% and 1.6% of the administered dose of tartrazine being excreted as sulfanilic acid and aminopyrazolone, respectively. Only a trace amount of intact tartrazine was detected in the urine [Honohan *et al*., 1977].

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

The melting point of FD&C Yellow No. 5 was calculated to be 350 °C using modeling software [MPBPVPWIN EPI Suite, 2000]. Substances of similar structure and molecular weight decompose on heating to temperatures >300 °C.

3.1.2 Boiling Point

The boiling point of FD&C Yellow No. 5 was calculated to be 870 °C [MPBPVPWIN EPI Suite, 2000]. Technically, data for this endpoint are not required given that this material is a solid and would likely decompose upon heating to elevated temperatures.

3.1.3 Vapor Pressure

The calculated vapor pressure for FD&C Yellow No. 5 has been reported to be 7.43 X 10-22 mm Hg at 25°C [MPBPVPWIN EPI Suite, 2000]. Given the high molecular mass of FD&C Yellow No. 5 (556.34) and the estimated Henry's law constant for azo dyes of 10^{-15} atm-m^3/mol it is highly unlikely that FD&C Yellow No. 5 would exhibit any significant (less than 0.001 mm Hg) vapor pressure. This is predicted by the MPBPVPWIN model. Based on these data, the vapor pressure is less than 1 X 10^{-20} mm Hg.

3.1.4 Octanol/Water Partition Coefficients

Log K_{OW} value for FD&C Yellow No. 5 is –10.17 [KOWWIN EPI Suite, 2000]. The experimental log K_{OW} value would be difficult to obtain by OECD methods given the large difference between water solubility and anticipated solubility in octanol. Based on the observations that FD&C Yellow No. 5 is freely soluble in water (200,000 mg/L) and

essentially insoluble in a relatively polar solvent like ethanol (10 mg/L) [Marmion, 1991], it is anticipated that the log K_{OW} value for this substances would exceed -6.0.

3.1.5 Water Solubility

FD&C Yellow No. 5 has a reported water solubility of 38,000 mg/L at 2 °C, 200,000 mg/L at 25 °C, and 200,000 mg/L at 60 °C [Marmion, 1991]. The solubility of FD&C Yellow No. 5 in 100% glycerol is 180,000 mg/L at 25 °C while the solubility in ethanol is reported to be 10 mg/L at 60 °C [Marmion, 1991, robust summary not included]. The solubility of FD&C Yellow No. 5 in octanol is expected to be less than 1 mg/L.

3.1.6 New Testing Required

None.

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

Direct and indirect photolysis experiments were conducted on the structurally related monoazo dye, FD&C Red No. 40[2], using two 15-watt low pressure lamps as the ultraviolet light source. Following 50 minutes of exposure to the lamps, FD&C Red No. 40 concentration decreased by 7% in the direct experiment. In the indirect experiment which used acetone as the sensitizer, the concentration of FD&C Red No. 40 decreased by 99% after 20 minutes [Pasin and Rickbaugh, 1991]. The calculated half-life for FD&C Yellow No. 5 in hydroxyl radical reactions is 3.5 hours [AOPWIN EPI Suite, 2000].

3.2.2 Stability In Water

FD&C Yellow No. 5 does not contain functional groups (*e.g.*, esters, amides, acetals, epoxides, lactones, *etc*.) that hydrolyze in water. The only potential reactivity in water would involve desulfonation of the aromatic sulfonic acid or its corresponding sulfonic acid salt. In aqueous acid (sulfuric acid), aromatic sulfonic acids desulfonate at temperatures of 100 to 175 ^{o}C. These conditions would not typically be encountered in the environment. Under environmental conditions, FD&C Yellow No.5 is ionized due to the presence of sulfonic acid and carboxylic acid functional groups exhibiting pKa values less than 4.0. Given their ionic nature, they are soluble and stable in water. Therefore, FD&C Yellow No. 5 and its corresponding salts are anticipated to be stable in water.

3.2.3 Biodegradation

The biodegradability of azo dyes ring-substituted with a carboxylic acid and two sulfonic acid groups consistently show that these substances are not absorbed onto activated

sludge and, therefore, are not biodegradable [Shaul *et al.*, 1990]. Incubation of 1.0 or 5.0 mg/L of a structurally related azo dye, (1-naphthalenesulfonic acid, 4-hydroxy-3-[(4-sulfo-1-naphthalenyl)azo]-, disodium salt)[3] with activated sludge from a sewage treatment plant revealed that the concentration of dye remained essentially constant in the influent flow, primary effluent, and activated sludge effluent. Essentially no azo dye was absorbed by activated sludge. Two other azo dyes ring-substituted with sulfonic acid groups (Acid Orange No. 10 and Acid Red No. 1) exhibited a similar behavior in these experiments. In an OECD 301C Guideline study D&C Red No. 9 (benzenesulfonic acid 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methyl, barium salt) was not readily biodegradable and was only 33% degraded after 21 days in a Zahn-Wellens test of inherent biodegradation (OECD SIDS 9th SIAM, 1999).

FD&C Yellow No. 5 was not predicted to be readily degradable by BIOWIN model calculations [AOPWIN EPI Suite, 2000].

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70 [ECOSAR EPI Suite, 2000]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log K_{OW}.

As expected, the model predicts that FD&C Yellow No. 5 is distributed completely to the water and soil compartments. Consistent with the extremely high water solubility and low log K_{OW} data, FD&C Yellow No. 5 showed no distribution to the fish compartment.

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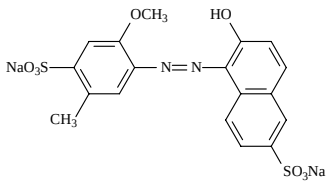

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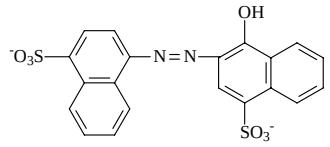


These data are consistent with ecotoxicity data for aromatic sulfonic acid derivatives that demonstrate essentially no absorption and toxicity to fish even at concentrations exceeding 1000 mg/L.

3.2.5 New Testing Required

None.

3.3 ECOTOXICITY

Introduction

A broad range of azo dyes that contain naphthalenesulfonic acid or benzenesulfonic acid substituents exhibit a low order of toxicity in aquatic species. Reactive Black 5 (diazo) containing 4 sulfonic acid groups shows a very low toxic potential in aquatic organisms (fish LC_{50} 100-500 mg/l; bacteria EC50 > 2,000 mg/l) as well as the hydrolysed dye (fish LC_{50} > 500 mg/l; Daphnia magna EC50 (48h) > 128 mg/l) (Hunger & Jung, 1991, IUCLID). The inability of azo dyes to react with various groups of vital organic materials, such as proteins and DNA, reduces the potential hazard considerably (ETAD, 1991).

Spencer (1984) has examined the effect of Aquashade (a mixture of Acid Blue 9 and Acid Yellow 23*) on the oxygen consumption of the crayfish *Orconectes propinquus* and has not found any effect at a concentration of 1 mg/l at an exposure of five days.

A survey of available fish toxicity data on over 3,000 commercially available organic dyes by ETAD member companies indicated that about 98% have a LC50 greater than 1 mg/l, a concentration at which coloring of a river normally would be observable. Dyes containing more than one sulfonic acid group show LC50 values > 100 mg/l.

No adverse effects on the carp (*Cyprinus carpio*) exposed to less than 10 mg/l of 30 water soluble (ionic) and 12 disperse dyes for 8 weeks (Brown, 1987). The table below provides a general overview of the testing results for a broad range of dyes including acid dyes and mordant dyes which contain sulfonic acid substituents

Zebra fish is susceptible to (in declining order) basic dyes > acid dyes > disperse dyes at a level less than 100 mg/l with acid dyes at >10 mg/l. For the other chemical classes, hydrolysed reactive, direct and mordant dyes with sulfonic acid groups, the LC50 is above 100 mg/l. The susceptibility to acid and basic dyes for fish is in agreement with the other findings (Clarke and Anliker, 1980).

The susceptibility of Daphnia resembles that of the zebra fish, but the order is different, basic > disperse > acid. The remaining chemical classes all show a LC50 above 100 mg/l. The study confirms the findings reported by other researchers (Hunger and Jung, 1991; IUCLID) that the reactive dyes and hydrolysed reactive dyes have a low toxic potential in aquatic organisms. One fish species (*Oryzias latipes*), exposed 48 hours to Acid Yellow 36, had a LC_{50} of 68 mg/l. For the remaining dyes, amongst them 5 acid, 6 direct and 2 solvent dyes, the LC50 was above and well above 100 mg/l. Apparently, the different fish species show very variable susceptibility.

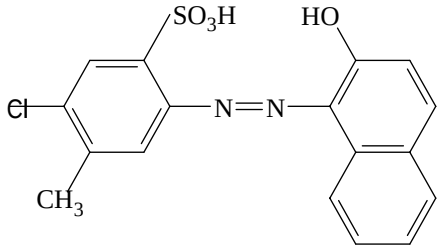
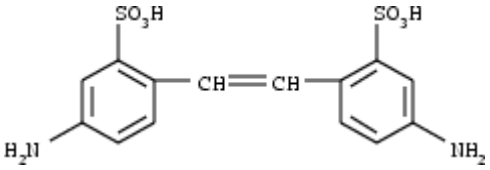
3.3.1 Acute Toxicity to Fish

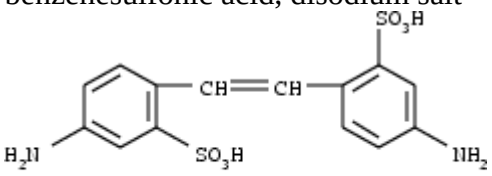
Based on input parameters for molecular weight (556.34), water solubility (200,000 mg/L at 25 °C), the calculated 96-hour LC50 for FD&C Yellow No. 5 is 1.14 X 10^{14} mg/L [ECOSAR EPI Suite, 2000] indicates a very low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Yellow No. 5 is to a large extent, a function of the presence of aromatic sulfonic acid (2) and carboxylic acid ring substituents. The presence of more than one aromatic sulfonic acid groups enhances water solubility and decreases absorption by aquatic species. Two structurally related substituted azo colorants containing naphthalene sulfonic acid and benzene sulfonic acid residues have been the subject of ecotoxicity studies in fish. Both exhibit a very low order of acute toxicity. The structural relative barium salt of 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid has been studied in two fish species (*Brachydanio rerio* and *Oryzias latipes*). The 96 hr- LC50 exceeded 500 mg/L, one in a semi-static test and the other in a static test (Hoechst AG, 1992). In other acute fish toxicity tests, the structurally related azo dye, 2-naphthalenecarboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt showed an 96-hr LC50=33 mg/L in Orange killifish (MITI, Japan, 1992). The fact that FD&C Yellow No. 5 contains two sulfonic acid groups and one carboxylic acid groups while structural analogs contain only one sulfonic acid group and one carboxylic acid group supports the conclusion that Yellow No. 5 is anticipated to exhibit even a lower toxicity than do the listed analogs. Both model daya

and data on these analogs support the conclusion that Yellow No. 5 exhibits a very low order (LC50 >100 mg/L) of toxicity for fish.

The presence of sulfonic acid residues in similar aromatic compounds limits absorption and concomitant toxicity in aquatic species. The extensive studies on the ecotoxicity of aromatic compounds containing two sulfonic acid groups indicate a very low order of toxicity to fish [Greim *et al.*, 1994]. Experimental LC50 values are available for stilbene sulfonic acids in which the N atom in the diazo dye is replaced by C. As indicated in Table 3 below, acute fish toxicity studies on salts of azo dyes and stilbene derivatives containing sulfonic acid derivatives show 96-hour LC50 value greater than 500 mg/L. Also, 48-hour and 72-hour LC50 concentrations of 200 and greater than 1000 mg/L, respectively have been reported [Greim *et al.*, 1994]. These values are consistent with calculated values.

Table 3

Name	Acute Toxicity to fish
barium salt of 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid 	96-hour LC50: >500 mg/L in *Brachydanio rerio* 96-hour LC50: >420 mg/L in *Oryzias latipes*
2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid 	48-hour LC50: 200 mg/L

2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt  • 2 Na	72-hour LC50: greater than 1000 mg/L

Given the high-calculated LC50 values from the ECOSAR model and the experimentally measured toxicity of azo dyes containing aromatic sulfonic acid substituents, no additional testing is requested.

3.3.2 Acute Toxicity to Aquatic Invertebrates

The calculated 48-hour LC50 value for FD&C Yellow No. 5 in *daphnids* is 5.25 X 10^{13} mg/L based on input parameters for molecular weight (556.34), and water solubility (200,000 mg/L at 25 °C), [ECOSAR EPI Suite, 2000] indicating a low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Yellow No. 5 is to a large extent, a function of the presence of aromatic sulfonic acid ring substituents (20 and a carboxylic acid substituent. This physiochemical property limits absorption and subsequent toxicity. Acute toxicity data on structurally related azo dyes containing sulfonic acid and carboxylic acid constituents support this conclusion.

Experimental data for the two azo colorants containing a benzene sulfonic acid or naphthalene sulfonic acid and a carboxylic acid substituents (barium salt of 5-chloro-2-[(2-hydroxyl-1-naphthenyl)azo]-4-methylbenzenesulfonic acid and 2-naphthalene carboxylic acid, [(4-methyl-2-sulfophenyl)azo], calcium salt) show low levels of toxicity in *Daphia magna.* In one study, an OECD 202 guideline study, the EC50 is reported to be 280 mg/L (EA, Japan, 1992). The extensive studies on the ecotoxicity of aromatic sulfonic acids also indicate a very low order of toxicity to aquatic invertebrates [Greim *et al.*, 1994]. An experimental 24-hour EC50 value with *Daphnia* for a stilbene sulfonic acid derivative, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, was greater than 100 mg/L [Greim *et al.*, 1994]. The fact that calculated values for FD&C Yellow No. 5 agree with experimental values for azo dyes containing sulfonic acid and carboxylic acid constituents and aromatic sulfonic acid derivatives, compounds that have limited absorption, supports the conclusion that FD&C Yellow No. 5 exhibits a low order of toxicity to aquatic invertebrates.

3.3.3 Acute Toxicity to Aquatic Plants

Based on input parameters for molecular weight (556.34), and water solubility (200,000 mg/L at 25 °C), the calculated 96-hour EC50 for FD&C Yellow No. 5 with green algae is 1.63 X 10^{13} mg/L [ECOSAR EPI Suite, 2000] indicating a very low order of acute toxicity. In a 96-hour algal chronic toxicity test, a sulfonic acid substituted azo dye, stimulated population growth (26.4%) compared to control (algal assay medium) [Greene and Baughman, 1996]. In fact, of the 46 dyes tested, only one, an anthraquinone dye, produced and measurable toxicity in terms of decreased algal growth rates. Given the low-predicted acute toxicity of FD&C Yellow No. 5 to aquatic plants and the stimulation of plant growth resulting from the addition of a structurally related azo dye in an experimental acute toxicity test, it is not recommended that additional tests be performed.

3.3.4 New Testing Required

None.

3.4 HUMAN HEALTH TOXICITY

3.4.1 Acute Toxicity

In reports submitted to the World Health Organization, the acute oral LD50 in mice was reported to be 12,750 mg/kg bw [National Institute of Hygienic Sciences of Japan, 1964]. In rats, the LD50 by intraperitoneal injection was reported to be 2,000 mg/kg bw and the LD50 by intravenous injection was reported to be 1,000 mg/kg bw [Deutsche Forschungsgemeinschaft, 1957].

3.4.2 *In vitro* and *In vivo* Genotoxicity

3.4.2.1 In vitro

FD&C Yellow No. 5 tested negative in reverse mutation assay using TA1535, TA1537, TA1538, TA98, and TA100 with and without metabolic activation [Chung *et al*., 1981; Ishidate *et al*., 1984; Muzzall and Cook, 1979]. In one chromosomal aberration test, FD&C Yellow No. 5 tested positive at concentrations up to 2,500 micrograms/mL (approximately 5 mM) without metabolic activation [Ishidate *et al*., 1984].

In an *in vitro* UDS assay using rat hepatocytes, FD&C Yellow No. 5 tested negative at concentrations up to and including 2 X 10^{-6} M [Kornbrust and Barfknecht, 1985].

3.4.2.2 In vivo

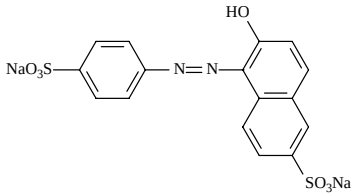
In an *in vivo* UDS assay, six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg/kg bw FD&C Yellow No. 5 *via* gavage. FD&C Yellow No. 5 did not induce unscheduled DNA synthesis at the dose level tested [Kornbrust and Barfknecht, 1985].

In a rodent micronucleus test, 10 ml/kg bw male rats were administered a single oral dose of 500 or 1000 mg/kg of the structurally related azo dye FD&C Yellow No. 6[4]. Bone marrow samples were taken at 24 and 48 hours later. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes at either time point in either species. [Westmoreland and Gatehouse, 1991].

3.4.3 Repeat Dose Toxicity

Groups of sixty male and sixty female mice each were administered 0, 0, 0.5, 1.5 or 5.0% FD&C Yellow No. 5 in the diet daily for 104 weeks. Animals were housed individually and fed the test diet *ad libitum*. Clinical observations were recorded twice daily, while detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for weeks 16-26 and monthly from week 26 until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (5.0%) and any animals with gross lesions or masses.

Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes, stomach, thymus, thyroid gland



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including parathyroid, trachea, and urinary bladder. Physical observations included hair loss, lacrimation, nasal discharge, staining of hair in the anogenital region and soft stools. None of these observations was attributed to administration of the test substance. Discolored urine and feces was reported at all treatment levels within one week of the study initiation. Mean body weights of both sexes were slightly lower than controls at the 5.0% treatment group for a number of sampling intervals, and male mice at the 1.5% treatment group were lower than controls for a number of sampling intervals. These differences were significantly lower in some intervals. Mean food consumption was significantly increased in male mice at the 5.0% treatment level. No statistically significant differences were reported for any of the hematological parameters. Common neoplastic, inflammatory, and degenerative lesions were reported amongst treated and control animals but were not considered to be treatment related. The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD&C Yellow No. 5. The no observable adverse effect level (NOAEL) of 5.0% providing an average daily intake of 8103 or 9753 mg/kg/day was established for male and female mice under the conditions of this study [Borzelleca and Hallagan, 1988b].

In a lifetime toxicity/carcinogenicity study, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD&C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents for 113 weeks. Animals were housed individually and fed the test diet *ad libitum*. Clinical observations were recorded twice daily with at least 5 hours between observations. Detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for the next 12 weeks and every 4 weeks thereafter until the end of the study. The intake of FD&C Yellow 5 was determined from body weight, food

consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on ten randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (2.0 or 5.0%) from each study and also on 10 rats randomly selected from each group for an interim sacrifice at 12 months. Histology was also performed on any animal with gross lesions or masses. Tissues examined included adrenal glands, aorta, blood smear, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, duodenum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes with epididymides, stomach, thymus, thyroid gland including parathyroid, trachea, urinary bladder, uterus.

During the *in utero* phase, there were no treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups. Slight decreases in body weight (4-5%) and slight increases in food consumption were noted in the F0 rats treated at dietary level of 5.0%. Two F0 female controls rats died during the *in utero* phase of the original study and one male and one female from the control and 5.0% group, respectively, died during the *in utero* phases of the high-dose study. There were no treatment related effects on pup survival.

In the F1 generation, a yellow tint was reported at all intake levels above 0.1%. At the 1.0% dietary level, group mean body weights at termination for both sexes were lower than the control animals, but the difference was only statistically significant for the females. In the high dose study (5.0% dietary level), group mean body weights were significantly lower in both sexes at termination. Food consumption was similar for control and treated animals at the 0.01, 1 or 2% dietary levels, but was slightly higher at the 5% level in the high-dose study, although not statistically significant. Hematological, clinical chemistry and urinalysis parameters did not differ significantly from the controls.

Necropsies at one year did not reveal any treatment-related gross or microscopic changes. At study termination, no treatment-related effects were reported on survival. No treatment-related changes were reported at gross necropsy. Histological evaluation revealed a variety of lesions, including neoplasms, present at similar incidences in control and treated animals. The authors considered the lesions to be spontaneous and not related to administration of the test material. The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD&C Yellow No. 5. A NOAEL of 5.0% providing an average daily intake of 2641 mg/kg/day and 3348 mg/kg/day for male and female rats, respectively, was reported under the conditions of this study [Borzelleca and Hallagan, 1988a].

3.4.4 Developmental Toxicity

In a guideline study performed by FDA, female Osborne-Mendel (FDA strain) rats (40-41 per group) were administered FD&C Yellow No. 5 *via* gavage at dose levels of 0, 60, 100, 200, 400, 600 or 1000 mg/kg bw/day for the first 19 days of gestation. On day 19, the animals were examined for gross abnormalities followed by euthanization. Caesarean sections were performed. The uterus was examined for presence and position of resorption sites and fetuses, number of corpora lutea and implantation sites. All live fetuses were promptly weighed, sexed, and examined. Crown-rump lengths were measured. Fetuses were divided and assigned to skeletal or soft tissue examination.

The authors reported no unusual behavior or external findings among the dosed females of any group. One female at the 60 mg/kg bw/day dose level died on gestation day 13 due to gavage difficulties. The mean daily food consumption of rats administered the 1000 mg/kg bw/day dose level was significantly greater than the controls. Initial body weight and maternal weight gain during gestation did not significantly differ between treated animals and controls. Pregnancy rate was similar among all groups.

No dose related findings were reported on fetal viability or fetal development. The incidence of sternebral variations was similar for all groups. The authors commented that the significant increase in food consumption observed in the highest dose group without a

corresponding effect on body weight indicated an effect on food utilization. The authors concluded that FD&C Yellow No. 5 was neither developmentally toxic nor teratogenic under the conditions of the study. The NOAEL for maternal and fetal toxicity was determined to be greater than 1000 mg/kg bw/day [Collins *et al*., 1990].

3.4.5 Reproductive Toxicity

In a lifetime toxicity/carcinogenicity study, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD&C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents for 113 weeks. During the *in utero* phase, there were no treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups [Borzelleca and Hallagan, 1988a].

3.4.6 New Testing Required

None.

3.5 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
C.I. Acid Yellow 23 CAS No. 1934-21-0	Calc	Calc	Calc	Calc	A

Chemical	Environmental Fate and Pathways			
	Photodegradation	Stability in Water	Biodegradation	Fugacity
C.I. Acid Yellow 23 CAS No. 1934-21-0	R, Calc	NA	R, Calc	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
C.I. Acid Yellow 23 CAS No. 1934-21-0	R, Calc	R, Calc	R, Calc

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
C.I. Acid Yellow 23 CAS No. 1934-21-0	A	A	A, R	A	A	A

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

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